SEED PROTEINS OF Ricinus communis.

II. ISOLATION AND COMPARATIVE CHARACTERIZATION OF THE ACID FORM OF RICIN

D. A. Khashimov, Kh. G. Alimov, and P. Kh. Yuldashev

UDC 665.117.4.093.5

An acid form of ricin – ricin T – with a molecular weight of 58 kDa, an isoelectric point, pI, of 7.0-7.1, and a sedimentation coefficient of 4.60 S has been isolated from the seeds of the Central Asian castor-oil plant Ricinus communis by gel filtration on Sephadex G-75 followed by ion exchange chromatography on DEAE- and CM-celluloses. The amino acid composition and the N-terminal amino acid residues of ricin T have been determined.

Several forms of the toxic protein that has been called by various authors toxalbumin [1, 2], phytotoxin [3], toxin [4, 5], RCA_{II} [6], ricin [7-15], ricin D and ricin E [16, 17], and ricin variant [18] have been isolated from the seeds of Ricinus communis L. from various growth sites. Among these it is ricin D that has been studied the most. In the present paper we report the isolation of an acid form of ricin from the seeds of the Central Asian castor-oil plant (castor beans) which we have called ricin T, and we give a characterization of it. Ricin T was obtained from a defatted flour of castor beans by a published method [16]. Gel filtration on Sephadex G-75 was used to purify the ricin. Separation was carried out in a 0.05 M Tris-HCl buffer having pH 8.0. Three fractions were obtained: G-1, G-2, and G-3, with yields of 30, 43, and 27%, respectively (Fig. 1).

Electrophoresis in 7.5% PAAG at pH 8.3 showed the presence in fraction G-2 of several protein components (Fig. 1). By separating fraction G-2 on DEAE-cellulose equilibrated with 0.005 M Tris-HCl buffer, pH 8.5, we obtained four main fractions: DE-1, DE-2, DE-3, and DE-4 (Fig. 2a) with yields of 18.2, 36.2, 20.4, and 25.2%, respectively. Fraction DE-1 was eluted with the initial buffer, and DE-2, DE-3, and DE-4 with buffer containing 0.025, 0.04, and 0.2 M NaCl, respectively. On disk electrophoresis, fraction DE-2 appeared in the form of three bands: one brightly colored and two minor ones (Fig. 2a). The further purification of fraction DE-2 was performed on CM-cellulose equilibrated with 0.005 M phosphate buffer, pH 6.5. The protein fractions were eluted with the initial buffer and then with buffer containing 0.01, 0.02, and 0.2 M NaCl, successively. Figure 2b, shows a profile of the chromatographic separation on CM-cellulose and an electrophoretogram of the main peak DE2-CM-1 eluted with buffer containing 0.01 M NaCl. Electrophoresis of fraction DE2-CM-1 showed only small amounts of impurities. The further purification of fraction DE2-CM-1 was carried out by chromatography on DEAE-cellulose (Fig. 2c), and it was then dialyzed against distilled water and lyophilized.

The homogeneity of the protein obtained (DE2-CM-1-DE-1), ricin T, was evaluated by disk electrophoresis, ultracentrifugation, and isoelectric focusing. Sedimentation analysis showed a single symmetrical peak with a sedimentation coefficient of 4.60 S. On isoelectric focusing in Ampholine supports in the pH range of 6-8, a pI value of the protein of 7.0-7.1 was obtained (Fig. 3). The molecular weight determined by the Weber–Osborn method [19] was 58,000 daltons.

The comparative characterization of the protein – ricin T – with ricins known from the literature is shown in Table 1 and 2.

As we see, the ricin T that we had isolated differed considerably in molecular weight, isoelectric point, and sedimentation coefficient from ricins D and E and ricin variant

Institute of the Chemistry of Plant Substances, Uzbek Academy of Sciences, Tashkent.
Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 883-887, November-December, 1987. Original article submitted April 20, 1987.

 0009-3130/87/2306-0738$12.50

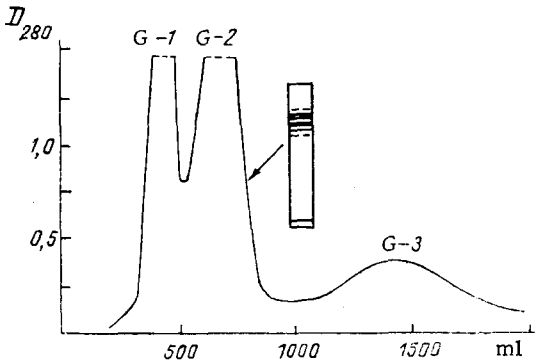

Fig. 1. Gel Chromatography of crude ricin on Sephadex G-75. Column 4 × 100 cm; rate 32 ml/h.

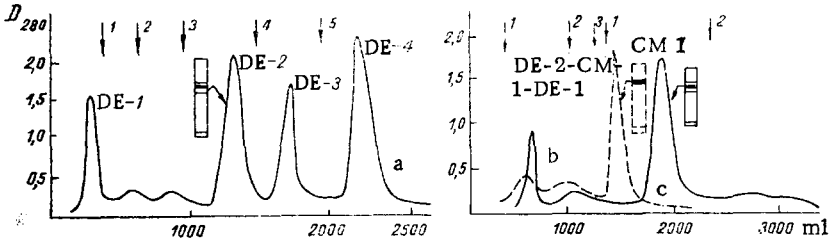

Fig. 2. Chromatography: a) of fraction G-2 on DEAE-cellulose; b) of fraction DE-2 on CM-cellulose; c) of fraction CM-1 on DEAE-cellulose. Column 2.5 × 30 cm; rate 20 ml/h.

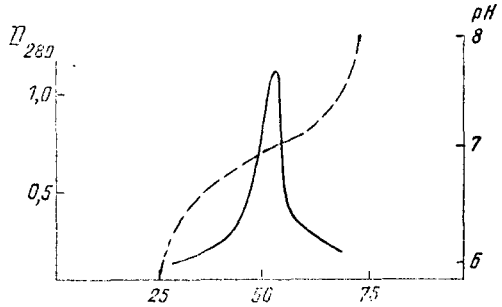

Fig. 3. Isoelectric focusing of ricin T on Ampholine supports in the pH interval of 6-8.

isolated previously [16-18]. A marked difference was observed in chromatography on DEAE-cellulose at pH 8.5 in 0.005 M Tris-HCl buffer (Fig. 2a). The peaks corresponding to the ricin D and ricin variant, which were eluted under the same conditions at 0.01, 0.013, and 0.015 M NaCl, were very slight in our case. These results show the more acidic nature of ricin T than of ricins D and E and ricin variant (see Table 1). A comparison of the amino acid compositions of ricins T, D, and E and ricin variant (see Table 2) showed that the amino acid composition of ricin T differed from ricins D and E and ricin varient with respect to the amounts of aspartic and glutamic acids, threonine, leucine, and isoleucine.

Thus, from the seeds of the Central Asian castor-oil plant we have isolated a new variety of ricin – T – with a molecular weight of 58,000 daltons, pI 7.0-7.1, sedimentation coefficient 4.60 S. Proteins corresponding to ricin D were absent or present in only very small amount in the seeds.

EXPERIMENTAL

Sephadex G-75 from Sweden, DE-32 and CM-32 celluloses from the United Kingdom, and Ampholines pH 3-10 and 6-8 from Sweden were used.

TABLE 1. Physical Constants of Isotoxins from the seeds of Ricinus communis

Fraction	Mol. wt.	Isoelectric point, pI	Sedim. coeff.	Literature
Ricin T	58000	7,0—7,1	4,60 S	
CII	63000	9		20
Ricin E	64000	8.8	4,45 S	17
Ricin D	60000	7,34	4,64 S	16
Ricin variant	57000	7.25	4,60 S	18
Toxin	55000			4,5
Ricin	65000			13
Ricin (fraction D)	65000	7,1		14
RCA II	60000			6
Ricin (fraction Q)	67000			15

TABLE 2. Amino Acid Compositions of Ricins T, D, and E and of Ricin Variant

Amino acid	Ricin T	Ricin D [16]	Ricin E [17]	Ricin variant [18]
Asp	62	59	63	59
Thr	36	35	39	36
Ser	39	37	33	39
Glu	49	46	45	46
Pro	25	26	28	26
Gly	35	36	39	36
Ala	38	37	41	37
1/2 Cys	10	12	9	12
Val	29	29	35	29
Met	6	6	7	6
Ile	36	35	39	34
Leu	44	42	49	44
Tyr	20	20	21	21
Phe	19	18	18	18
Lys	9	9	12	9
His	6	6	7	6
Arg	32	32	37	34
Trp	8	9	8	8
Sum of the residues	495	493	522	500
N-Terminal amino acids	Ile/Ala	Ile/Ala	Ile/Ala	Ile/Ala

The castor beans freed from husks (500 g) were ground, extracted with cooled petroleum ether, and washed on a Büchner funnel cooled with ether until the oil had been eliminated completely, and were then dried in the air. The yield of defatted flour was 150 g (30%).

Gel chromatography was performed by a published method [16] on Sephadex G-75, 700 mg of total protein dissolved in 5 ml of 0.05 M Tris-HCl buffer, pH 8.0, being fractionated. The rate of elution was 32 ml/h. The fractions obtained (8 ml each) were subjected to spectrophotometry on an SF-16 at 280 nm. The fractions relating to a single peak were combined, dialyzed against water, and freeze-dried. The yield of peak I was 200 mg, of peak II 250 mg, and of peak III 150 mg.

Column Chromatography on DEAE-Cellulose. A solution of 1 g of fraction G-2 in 50 ml of 0.005 M Tris-HCl buffer, pH 8.5, was deposited on a column equilibrated with the same buffer. Elution was performed in steps with the initial buffer containing 0.01, 0.015, 0.025, 0.04, and 0.2 M NaCl.

Column Chromatography on CM-Cellulose. Fraction DE-1 (700 mg) was dissolved in 10 ml of 0.005 M phosphate buffer, pH 6.5, and was eluted with the same buffer containing 0.01, 0.013, 0.025, and 0.5 M NaCl. The volume of samples taken was 5 ml every 15 min.

The chromatography of fraction DE-2-CM-1 on DEAE-cellulose was carried out under the same conditions as those described above.

Disc electrophoresis in PAAG was performed by a known method [21] at a voltage of 300 V, 30 mA, in Tris-glycine buffer, pH 8.3, for 4 h. The gels were stained with a 0.1% solution of Coomassie blue in methanol—water—acetic acid (5:5:1). The residues of the dye were washed out with a 7% solution of acetic acid.

The molecular weight of ricin T was determined by Andrews' method [22]. Lactate dehydrogenase (mol. wt. 135,000), hemoglobin (mol. wt. 70,000), catalase (mol. wt. 60,000) pepsin (mol. wt. 33,000), trypsin (mol. wt. 24,000) and cytochrome C (mol. wt. 12,000) were used as markers.

Ultracentrifugation. A solution of 10 mg of the protein in 1 ml of 0.1 M sodium acetate buffer, pH 5.0, was centrifuged in a MOM-3170 ultracentrifuge. The sedimentation coefficient was calcualted as in [23].

The isoelectric point of the protein was determined by a published method [24] on a LKB-2117 Multiphor instrument (Sweden). Ampholine electrophoresis was carried at pH 6-8 on Sephadex G-75 for 48 h.

The amino acid composition was determined by the method of Moore and Stein [25] on a LKB-4101 amino acid analyser (Sweden). N-terminal amino acids were determined by the dansyl method [26].

SUMMARY

1. A new variety of ricin has been isolated from the seeds of the Central Asian castor-oil plant Ricinus communis – ricin T – with a molecular weight of 58,000 daltons, an isoelectric point, pI, of 7.0-7.1, and a sedimentation coefficient of 4.60 S.

2. The amino acid composition and the N-terminal amino acids of the protein molecule have been determined.

LITERATURE CITED

1. É. LeBreton and Y. Moulé, C. R. Acad. Sci., Paris, 225, 151 (1947).
2. A. Lugnier and G. Dirheimer, C. R. Acad. Sci., Paris, 273, 704 (1971).
3. J. M. Kingsbury, Poisonous Plants of the United States and Canada, Prentice-Hall, Englewood Cliffs, New Jersey (1964), p. 626.
4. E. Waldschmidt-Leitz and L. Keller, Z. Physiol. Chem., 351, 990 (1970).
5. L. G. Gurtler and H. J. Horstmann, Biochem. Biophys. Acta, 295, 582 (1973).
6. G. L. Nicholson, J. Blaustein, and M. E. Etzler, Biochemistry, 13, 196 (1974).
7. É. LeBreton and Y. Moulé, Bull. Chem. Biol., 31, 94 (1949).
8. M. Mourgue, R. Dokhan, and J. Renaud, Bull. Soc. Chem. Biol., 40, No. 11, 1453 (1956).
9. M. Funatsu and G. Funatsu, J. Agric. Chem. Soc. Jpn., 33, No. 6, 461 (1958).
10. G. R. Waller, K. E. Ebner, and R. A. Scroggs, Proc. Soc. Exp. Biol. and Med., 121, 685 (1966).
11. J. Y. Lin, K. Y. Tsern, and T. C. Tung, Hsueh Hui Tsa Chih. 68, No. 10, 518 (1969).
12. G. Funatsu and M. Funatsu, Jpn. J. Med. Soc. Biol., 23, 342 (1970).
13. S. Olsnes, K. Refsnes, and A. Phil, Nature (London), 249, 627 (1974).
14. S. Olsnes, Methods Enzymol., 50, 330 (1978).
15. T. T.-S. Lin and S. S.-L. Li, Eur. J. Biochem., 105, No. 3, 453 (1980).
16. M. Funatsu, G. Funatsu, M. Ishiguro, S. Nanno, and K. Hara, Proc. Jpn. Acad., 47, 713 (1971).
17. T. Mise, G. Funatsu, M. Ishiguro, and M. Funatsu, Agr. Biol. Chem., 41, No. 10, 2041 (1977).
18. M. Ishiguro, M. Tomi, G. Funatsu, and M. Funatsu, Toxicon, 14, No. 3, 157 (1976).
19. K. Weber and M. Osborn, J. Biol. Chem., 244, 4406 (1969).
20. L. Genand, J. Guillot, G. Bétail, and M. Coulet, J. Immunol. Meth., 49, 323 (1982).
21. L. Ornstein and B. J. Davis, Ann. N. Y. Acad. Sci., 121, 321 (1964).
22. P. Andrews, Biochem. J., 96, No. 4, 595 (1965).
23. H. K. Schachmann, Biochemistry, 2, 887 (1963).
24. O. Vestereg and S. Harry, Acta Chem. Scand., 20, 820 (1966).
25. D. H. Spackman, S. Moore, and W. H. Stein, Anal. Chem., 30, 1190 (1958).
26. T. Devenyi and J. Gergely, Amino Acids, Peptides and Proteins, Elsevier, New York (1974) [Russian translation, Moscow (1976), p. 274].